

REMARKS

Formal request for interview

This Response is believed clearly to place this application in condition for allowance. Nevertheless, the Examiner is respectfully requested to contact Applicants' undersigned attorney, following receipt of this Response, in order to schedule an interview. The interview will be for the purpose of resolving any outstanding issues (should the Examiner believe that issues remain outstanding) and advance the case to allowance. The undersigned attorney further requests that the Examiner's supervisor, Michael G. Lee, participate in the interview.

Accordingly, this paper should be treated as a formal request for an interview to take place before the mailing of any next Action.

Status of claims

- Claims 7, 9-11, and 13-22 remain pending in this application.
- Claims 7, 21, and 22 are independent.

The rejection under 35 U.S.C. § 112

- Claims 7 and 22, and therefore dependent claims 9-11 and 13-20, were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

At page 2 of the Office Action, the Examiner states:

Regarding claims 7 and 22, the limitation citing "wherein at least one of the perforations has an elongate cross section with **a minimum and a maximum diameter**" is deemed vague and indefinite since the claim language does not set forth a clear understanding as to how any shape may have a minimum and a maximum diameter. A circle has only one diameter. An elongated shape such as a rectangle has no diameter. An elongated shape such as an oval has no diameter.

For examination purposes, the shape of the elongate cross section with a diameter will be interpreted as a rectangle.

(Emphasis in original.)

Thus, the Examiner deems the recitation of "an elongate cross section with a minimum and a maximum diameter" in claims 7 and 22 to be vague and indefinite. In particular, the Examiner states: "*An elongated shape such as a rectangle has no diameter. An elongated shape such as an oval has no diameter.*" Applicants respectfully submit that this is incorrect, and hereby traverse the Examiner's comments.

Applicants attach a copy of the definition of the word "diameter" according to the venerable *Webster's Dictionary, 2nd edition*. According to that definition, a diameter is, *inter alia*, "Any cord passing through the center of a figure or body" (see point 1 under "diameter"), or "The length of a straight line through the center of an object" (see point 2). That definition is not restricted to a circle at all.

Similarly, the *American Heritage Dictionary* defines "diameter" as "A straight line segment passing through the center of a figure, especially of a circle or sphere, and terminating at the periphery." Thus, the term is not only applicable to circles or spheres. This is illustrated by the annexed page from *Ask.com*.

For the example of ellipses, Applicants refer to the annexed page from the *Math Open Reference* (mathopenref.com) that shows the “major” and “minor” axes of an ellipse, and states that “the major and minor axes of an ellipse are diameters (lines through the center) of the ellipse.” The *Math Open Reference* goes on to define the major axis of the ellipse as the “longest” diameter of the ellipse and the minor axis of the ellipse as the “shortest” diameter of the ellipse.

Algebra.com also has an entry relating to ellipses, of which Applicants include (for brevity) the first two pages only. Again, the smallest and largest diameters of the ellipse are discussed and shown. In particular, *Algebra.com* states:

Unlike a circle, whose diameter is the same length regardless of how you orient it, the ellipse has different length diameters depending on how you orient them.

The largest diameter falls on what is called the major axis.

The smallest diameter falls on what is called the minor axis.

In between, the diameters have varying lengths.

(Emphasis added.)

Applicants, still further, note the following additional references for the Examiner’s attention:

- WO 2004/033592 (page 5 attached). Paragraph 0019 mentions "minimum diameter and maximum diameter" for an ellipse.

► http://www.j3d.org/matrixfaq/curvfaq_latest.html (pages 10 and 11, attached). This reference states:

Q16. What is an ellipse?

An ellipse is a closed curve similar to a circle. However, the radius varies between a minimum diameter and a maximum diameter.

(Emphasis added.)

► <http://cercor.oxfordjournals.org/cgi/reprint/16/7/990.pdf> (pages 990-992, attached). See page 992, right column, where the terms "minimum diameter" and "maximum diameter" are used for fairly complicated, non-circular objects (neurons).

Accordingly, Applicants submit that a person having ordinary skill in the art is well able to understand the terms "minimum diameter" and "maximum diameter" as recited in claims 7 and 22. Applicants also note the description of these terms in the present application with reference to the specification and drawings, for example the specification at page 4, lines 14-21, and Figs. 3 and 4¹:

The cross sections of holes 5a and 5b in the embodiment of Figs. 3 and 4 are of equal elongate shape, but rotated in respect to each other by an angle of 90 degrees. Each hole is of roughly ellipsoidal cross section having a minimum diameter d1 and d1' and a maximum diameter d2 and d2', respectively. The minimum diameter d1 of hole 5a is substantially parallel to the maximum diameter d2' of hole 5b and vice versa. (Emphasis added.)

¹It is of course to be understood that the references to various portions of the present application are by way of illustration and example only, and that the claims are not limited by the details shown in the portions referred to.

Thus, given all that is presented above, Applicants submit that the recitations "minimum diameter" and "maximum diameter" are not vague and indefinite.

For at least the foregoing reasons, withdrawal of the rejection under 35 U.S.C. § 112, second paragraph, is respectfully requested.

The rejections under 35 U.S.C. § 102

- Claims 7, 9-11, and 13-22 were rejected under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent Application Publication No. US 2003/0038423 to Turner.

Applicants submit that independent claims 7, 21, and 22, together with the claims dependent therefrom, are patentably distinct from Turner for at least the following reasons.

First, claims 7, 21, and 22 all claim a "security document." The Examiner deems that such a security document is anticipated by the hexagonal toy disclosed by Turner.

MPEP 2111 states that claims must be given their "broadest **reasonable** interpretation," and one that is consistent with the specification and with the interpretation that those skilled in the art would reach. (Emphasis added.) Applicants can find nothing "reasonable" about the Examiner's interpretation.

MPEP 2111 explicitly states that the words should be given their "broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art." No one skilled in the art would understand the term "security document" as covering Turner's toy. The Turner toy is not a document, let alone a security document.

Applicants note that the Examiner even asserts that Turner's toy is a passport or a banknote (see the rejection of claim 19). Applicants wonder which bank or customs would accept that toy as a banknote or a passport. No skilled person would consider that toy to be a security document, let alone a passport or a banknote. Turner does not teach or suggest a security document, let alone a passport or a banknote.

Turner does not teach or suggest "a security feature," as recited in claim 7. Turner discloses an educational toy/game. Turner has nothing at all to do with bank notes, passports, or security documents. Turner does not teach or suggest "at least two of the perforations have different cross sections... wherein said cross sections have equal areas," as recited in claim 7.

It is well settled that:

**TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH
EVERY ELEMENT OF THE CLAIM MPEP 2131 (emphasis original).**

Moreover, Applicants note 37 C.F.R. 1.104(c)(2), as quoted in MPEP 706, which recites, *inter alia*:

...When a reference is complex or shows or describes inventions other than that claimed by the applicant, the particular part relied on must be designated as nearly as practicable. The pertinence of each reference, if not apparent, must be clearly explained and each rejected claim specified.

However, while the Office Action does reference all of the claims by number in rejecting them, it is respectfully submitted that the reasoning set forth in the Office Action (especially with respect to the dependent claims) amounts in large part to a general allegation that the claims are unpatentable, without more clearly explaining the pertinence of the reference, including the particular parts relied on, with respect to each rejected claim. Accordingly, it is submitted that any next Action, if it is not an allowance, should be made non-final.

For at least the foregoing reasons, independent claims 7, 21, and 22, and the claims depending therefrom, are seen to be clearly allowable over Turner.

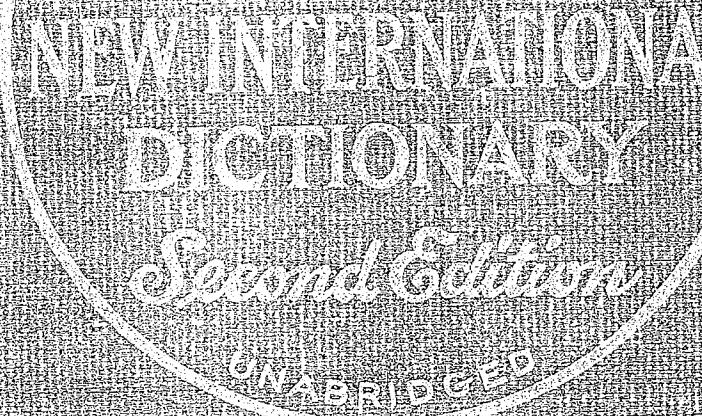
Conclusion

In view of the foregoing remarks, Applicants respectfully request favorable reconsideration and early passage to issue of the present application.

Respectfully submitted,

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**Definitions of 'diameter'** (dī-ämī-tər) ▶▶

Dictionary.com · The American Heritage® Dictionary - (3 definitions)

[Middle English *diametre*, from Old French, from Latin *diametruS*, from Greek *diametros* (*grammē*), diagonal (line), *dia-*, dia-, + *metron*, measure.]

(noun)1. *Mathematics*

- a. A straight line segment passing through the center of a figure, especially of a circle or sphere, and terminating at the periphery.
- b. The length of such a segment.

2. Thickness or width.

- 3. A unit for measuring the magnifying power of a microscope lens or telescope, equal to the number of times an object's linear dimensions are apparently increased.

(derivatives)

di·ame·trial

adjective

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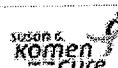
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Search Dictionary

Q
**The American Heritage® Dictionary of the English Language, Fourth Edition**

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Major / Minor axis of an ellipse

Major axis: The longest diameter of an ellipse.
Minor axis: The shortest diameter of an ellipse.

Try this Drag any orange dot. The ellipse changes shape as you change the length of the major or minor axis. (If there is no image below, see support page.)

Major axis →

Minor axis

Drag any orange dot

Run Full screen Hide details RESET

The major and minor axes of an ellipse are diameters (lines through the center) of the ellipse. The major axis is the longest diameter and the minor axis the shortest. If they are equal in length then the ellipse is a circle. Drag any orange dot in the figure above until this is the case.

Each axis is the perpendicular bisector of the other. That is, each axis cuts the other into two equal parts, and each axis crosses the other at right angles.

The focus points always lie on the major (longest) axis, spaced equally each side of the center. See Foci (focus points) of an ellipse

Calculating the axis lengths

Recall that an ellipse is defined by the position of the two focus points (foci) and the sum of the distances from them to any point on the ellipse. (See Ellipse definition and properties). Referring to the figure on the right, if you were drawing an ellipse using the string and pin method, the string length would be $a+b$, and the distance between the pins would be f .

The length of the minor axis is given by the formula:

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About Theo:

This lesson provides an overview of equations involving an ellipse.

REFERENCES

<http://en.wikipedia.org/wiki/Ellipse>
<http://www.analyzemath.com/EllipseEq/EllipseEq.html>
http://www.algebralab.org/lessons/lesson.aspx?file=Algebra_conics_ellipse.xml
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<http://www.physicsforums.com/showthread.php?t=116790>
<http://www.mathwarehouse.com/ellipse/is-circle-an-ellipse.php>
<http://www.mathopenref.com/constellipse1.html>

DEFINITION OF AN ELLIPSE

An ellipse has a center called the vertex of the ellipse.

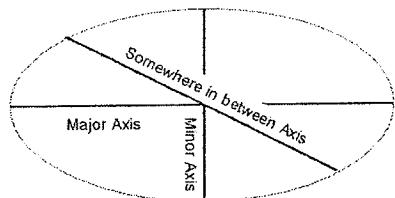
Unlike a circle, whose diameter is the same length regardless of how you orient it, the ellipse has different length diameters depending on how you orient them.

The largest diameter falls on what is called the major axis.

The smallest diameter falls on what is called the minor axis.

In between, the diameters have varying lengths.

A picture of what that looks like is shown below:



While the circle has a center, the ellipse has a center plus 2 focal points.

These 2 focal points lie on the major axis.

They lie on each side of the center of the ellipse and are equally distant from that center.

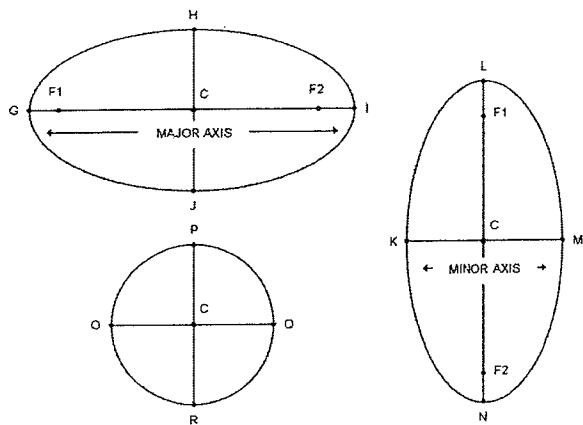
PICTURE OF A CIRCLE AND AN ELLIPSE

A picture of a circle and a horizontal ellipse and a vertical ellipse are shown below.

The circle is on the lower left. The horizontal ellipse is on the upper left. The vertical ellipse is on the right.

The circle is a special form of an ellipse as will be seen later in this lesson. The point C is the center of the circle. The points [O,P,Q,R] are on the circumference of the circle. The line segments [OQ,PR] are both the diameters of the circle and the vertical and horizontal axes of the circle. They are all the same length. The line segments

$[OC, PC, QC, RC]$ are all radii of the circle. They are all the same length. The circumference of the circle is formed by rotating any one of these radii about the center of the circle for 360 degrees.



The ellipse is an elongated circle. The point C is the center of the ellipse. The points $[F_1, F_2]$ are the focal points of the ellipse. It has a major axis and a minor axis. The length of the major axis is greater than the length of the minor axis. If the major axis is horizontal, then the ellipse is called a horizontal ellipse. If the major axis is vertical, then the ellipse is called a vertical ellipse. The horizontal ellipse in the upper left side of the above diagram has the points $[G, H, I, J]$ on its circumference. The vertical ellipse in the right side of the above diagram has the points $[K, L, M, N]$ on its circumference. The horizontal ellipse major axis = line segment GI and minor axis = line segment HJ . The vertical ellipse major axis = line segment LN and minor axis = line segment KM . The major axis forms the longest diameter of the ellipse. The minor axis forms the shortest diameter of the ellipse.

RADI AND CIRCUMFERENCE OF AN ELLIPSE

With a circle, you have a radius that revolves around the center of the circle to form the circumference of the circle.

With an ellipse, you have 2 radii working together to form the circumference of the ellipse.

The length of each of the radii can vary, but the sum of their lengths is always equal to a constant.

That constant is equal to the length of the major axis.

The following picture shows you what this means:

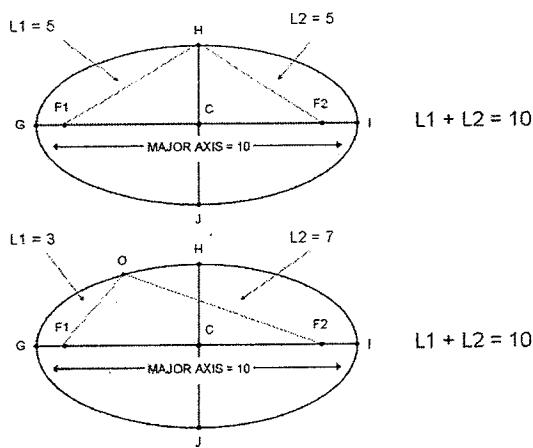
In the top ellipse:

The line segment between F_1 and H (shown as L_1 in the diagram) = 5 and the line segment between F_2 and H (shown as L_2 in the diagram) = 5.

The sum of their lengths is equal to 10 which is the length of the major axis.

Point F_1 and F_2 are the focal points of this ellipse.

Point H is any point on the surface of the ellipse which just happens to be equidistant from F_1 and F_2 because it lies on the intersection of the minor axis with the surface of the ellipse.



In the bottom ellipse:

The line segment between F_1 and O (shown as L_1 in the diagram) = 3 and the line segment between F_2 and O (shown as L_2 in the diagram) = 7.

The sum of their lengths is equal to 10 which is the length of the major axis.

Point F_1 and F_2 are the focal points of this ellipse.

Point O is any point on the surface of the ellipse. It could coincide with point G or I or H or J , or be anywhere else on the surface of the ellipse. The sum of the distances between F_1 and O , and between F_2 and O will always be a constant that is equal to the length of the major axis.

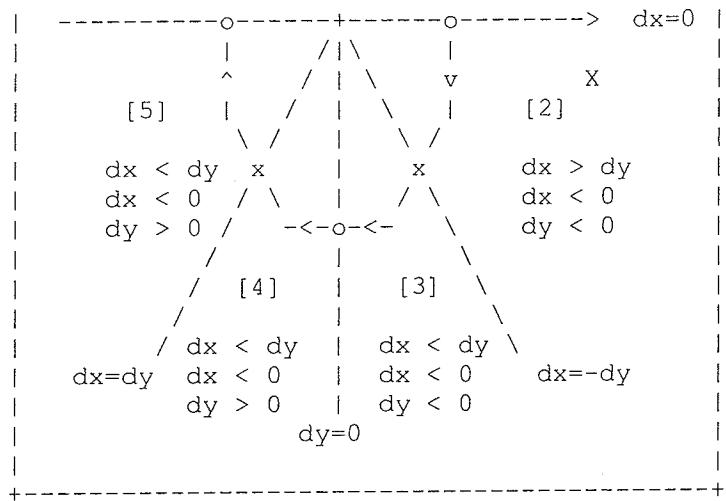
invention can give a lighter lube with yields similar to those obtained over ZSM-48 alone.

[0017] Preferably, wax feed is first passed over a ZSM-48 catalyst. The resulting intermediate product is then passed over a single Zeolite Beta catalyst to form the final lube. These first and second stages can be separated or preferably are integrated process steps (e.g., cascaded).

[0018] The unidimensional molecular sieve catalyst with near-circular pore structures does most of the dewaxing. The pores are smaller than in large pore molecular sieves thereby excluding bulkier (e.g., highly branched) molecules. Unidimensional means that the pores are essentially parallel to each other.

[0019] The pores of the catalyst have an average diameter of 0.50 nm to 0.65 nm wherein the difference between a minimum diameter and a maximum diameter is ≤ 0.05 nm. The pores may not always have a perfect circular or elliptical cross-section. The minimum diameter and maximum diameter are generally only measurements of an ellipse of a cross-sectional area equal to the cross-sectional area of an average pore. The pores can alternatively be defined by finding the center of the pore cross-section and using half of the minimum diameter and half of the maximum diameter to sweep an average cross-sectional pore shape from the center.

[0020] The preferred unidimensional molecular sieve catalyst is an intermediate pore molecular sieve catalyst of which the preferred version is ZSM-48. U.S. Patent 5,075,269 describes the procedures for making ZSM-48 and is incorporated by reference herein. ZSM-48 is roughly 65% zeolite crystal and 35% alumina. Of the crystals, at least 90%, preferably at least 95%,



Expressing each line has the following equation:

$$Ax + By + C = 0$$

generates the following table:

Equation	A	B	C
dx=dy	X-Y=0	1	-1
dx=0	X=0	1	0
dx=-dy	X+Y=0	1	1
dy=0	Y=0	0	1

Each octant has the following attributes:

Octant #	dx	dy	dx ?? dy	start	end
0	> 0	< 0	dx > dy	dy= 0	dx= dy
1	> 0	< 0	dx > dy	dx= dy	dx= 0
2	< 0	< 0	dx > dy	dx= 0	dx=-dy
3	< 0	< 0	dx < dy	dx=-dy	dy= 0
4	< 0	> 0	dx < dy	dy= 0	dx= dy
5	< 0	> 0	dx < dy	dx= dy	dx= 0
6	> 0	> 0	dx < dy	dx= 0	dx=-dy
7	> 0	> 0	dx > dy	dx=-dy	dy= 0

ELLIPSES

=====

Q16. What is an ellipse?

An ellipse is a closed curve similar to a circle. However, the radius varies between a minimum diameter and a maximum diameter.

In effect, an ellipse can be considered to be a squashed or stretched circle.

An axis-aligned ellipse can be specified as a centre (c, d) and axis radii (r, s)

These can be combined to give the equation:

$$\frac{(x-c)^2}{r^2} + \frac{(y-d)^2}{s^2} - 1 = 0$$

Expnding out this expression will generate an equation identical to that of a circle. However, the values of A and B are not guaranteed to be equal to one.

The algebraic expression is:

$$Ax^2 + By^2 + Cx + Dy + Exy + F = 0$$

where A, B, C, D, E and F are constant terms.

Q17. How do I calculate the Y coordinate if only the X coordinate is known?

Given the equation:

$$[1] Ax^2 + By^2 + Cx + Dy + Exy + F = 0$$

Then the known value of x or y is defined as:

$$[2] x = X$$

$$y = Y$$

Substituting [2] into [1] gives:

$$[3] AX^2 + BY^2 + CX + DY + EXY + F = 0$$

$$Ax^2 + BY^2 + Cx + DY + ExY + F = 0$$

Moving all constant terms to the end of the equation gives:

$$[4] BY^2 + DY + EXY + AX^2 + CX + F = 0$$

$$Ax^2 + Cx + ExY + BY^2 + DY + F = 0$$

Converting these into quadratic equations gives:

$$Ry^2 + Sy + T = 0 \quad Rx^2 + Sx + T = 0$$

where:

$$R = B$$

$$R = A$$

$$S = D + EY$$

$$S = C + EY$$

$$T = AX^2 + CX + F \quad T = BY^2 + DY + F$$

These values can be solved using quadratic equations.

Dendritic Size of Pyramidal Neurons Differs among Mouse Cortical Regions

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Neocortical circuits share anatomical and physiological similarities among different species and cortical areas. Because of this, a 'canonical' cortical microcircuit could form the functional unit of the neocortex and perform the same basic computation on different types of inputs. However, variations in pyramidal cell structure between different primate cortical areas exist, indicating that different cortical areas could be built out of different neuronal cell types. In the present study, we have investigated the dendritic architecture of 90 layer II/III pyramidal neurons located in different cortical regions along a rostrocaudal axis in the mouse neocortex, using, for the first time, a blind multidimensional analysis of over 150 morphological variables, rather than evaluating along single morphological parameters. These cortical regions included the secondary motor cortex (M2), the secondary somatosensory cortex (S2), and the lateral secondary visual cortex and association temporal cortex (V2L/TeA). Confirming earlier primate studies, we find that basal dendritic morphologies are characteristically different between different cortical regions. In addition, we demonstrate that these differences are not related to the physical location of the neuron and cannot be easily explained assuming rostrocaudal gradients within the cortex. Our data suggest that each cortical region is built with specific neuronal components.

Keywords: Circuit, Lucifer Yellow, PCA, cluster analysis

Introduction

The search for guiding principles to understand the function of the cortical circuit has a long history. Cajal devoted many pages to speculations on potential functions that could be implemented by the anatomical pattern of neuronal morphologies and axonal innervation observed (Ramón y Cajal, 1899). His disciple Lorente de Nò described more than a hundred types of cortical neurons in mouse temporal cortex (Lorente de Nò, 1922) and characterized cortical circuits as vertical chains of neurons (Lorente de Nò, 1949). Based on electrophysiological recordings, Mountcastle, and later Hubel and Wiesel, proposed the columnar hypothesis, by which the neocortex would be composed of repetitions of one basic modular unit, and argued that the same basic cortical computation could be performed by a cortical module in different cortical areas (Hubel and Wiesel, 1974, 1977; Mountcastle, 1982, 1997). Thus, the task of understanding cortical function could be reduced to deciphering the basic 'transfer function' that that module performs on any input. The differences in function among different cortical areas would then be explained by the difference in the inputs they receive, rather than by intrinsic differences on cortical processing among areas.

In more recent times, Douglas and Martin have termed this idea the 'canonical microcircuit' hypothesis and have proposed

a series of basic circuit diagrams based on anatomical and electrophysiological data (Douglas *et al.*, 1989, 1995; Douglas and Martin, 1991, 1998, 2004). According to their hypothesis, the common transfer function that the neocortex performs on inputs could be related to the amplification of the signal (Douglas *et al.*, 1989) or a 'soft' winner-take-all algorithm (Douglas and Martin, 2004). These ideas agree with the recurrent excitation present in cortical tissue which could then exert a top-down amplification and selection on thalamic inputs (Douglas *et al.*, 1995).

There are many arguments in favor of a canonical microcircuit. Besides the electrophysiological evidence based on receptive fields, the anatomical presence of vertical chains of neurons defining small columnar structures has been noted since Lorente (Lorente de Nò, 1949). According to him, myelin stains show vertical bundles of pyramidal cell axons. Similar bundles of apical dendrites have been noticed by a number of authors using a variety of staining methods (Fleischauer, 1972; Fleischauer and Detze, 1975; Escobar *et al.*, 1988; Peters and Yilmaz, 1993; Peters and Walsh, 1972). These structural modules appear in many different regions of the cortex in many different species (e.g. Buxhoeveden *et al.*, 2002). In addition, support for the idea of canonical microcircuits has come from the basic stereotyped developmental program that different cortical regions (and cortices from different species) share (Purves and Lichtman, 1985; Jacobson, 1991). Like in other parts of the body, it is possible that the neocortex arose by a manifold duplication of a similar circuit module. The relatively short evolutionary history of the neocortex, together with the prodigious increase in size it has experienced in mammals, makes this idea appealing. Also, all cortices of all animals develop through a very stereotypical sequence of events, from neurogenesis in the ventricular zone, to migration along radial glia, depositing of neuroblasts in cortical layers and emergence of axons, dendrites and dendritic spines. These events occur in some cases with nearly identical timing in different parts of the cortex and in different animals, so it is not unreasonable to argue that they result in the assembly of an essentially identical circuit. Nevertheless, important differences in the specification of cortical areas have also been noted (Rakic, 1988). Finally, it is still unclear how much of the connectivity matrix is determined by early developmental events, and how much could be locally regulated or even controlled by activity-dependent Hebbian rules (Katz and Shatz, 1996). In this respect, transplantation experiments have indicated that axons from the visual pathway, when rerouted to the auditory or somatosensory areas, generate in the host neurons receptive properties which are similar to those found in visual cortex (reviewed in Sur, 1993; Frost, 1999). These data could be interpreted as supportive of the idea

of canonical microcircuits, but could also be explained as the product of developmental plasticity mediated by novel axons or axonal activity. A final argument in support for a canonical microcircuit comes from the stereotypical laminar and columnar input-output organization of the cerebral cortex (Gilbert and Wiesel, 1979; Jones, 1981).

On the other hand, there are also compelling reasons against this hypothesis. It is hard to imagine that there is a common denominator in all the different computational problems that the cortex is solving. In some cases, these problems appear mathematically irreducible even in their basic dimensionality, such as three-dimensional visual processing, as compared with auditory speech perception, for example. Also, the exact nature of the structure of the cortical modules is hard to define. Anatomical techniques do not reveal any clear borders between modules, and physiological approaches show instead, like in the primate primary visual cortex, a combination of maps superimposed onto one another with different metrics, such as orientation, ocular dominance, or spatial frequency (Bartfeld and Grinvald, 1992), although perhaps a more basic metric could underlie them (Basole *et al.*, 2003).

Furthermore, if evolution was duplicating circuit modules in different cortical areas or in the cortex of different animals, it would be expected that a canonical microcircuit, in the strict sense, would be built with the same components. Thus, the neuronal cell types and connections between these neurons should be very similar or even identical. In this respect, although it is generally agreed that cortical areas have the same complement of neuronal cell types (e.g. Rockel *et al.*, 1980), in some cases there are distinct types of neurons which are only found in particular cortical areas or species, such as the Meynert and the Betz giant pyramidal neurons, or certain types of spindle neurons and double bouquet cells (Nimchinsky *et al.*, 1999; DeFelipe *et al.*, 2002; Ballesteros-Yáñez *et al.*, 2005). In addition, recent work has demonstrated that the most typical and abundant neuron in cortex, the pyramidal cell, sampled from different areas of different primate species, has quantitative differences in the size of the dendritic arbor and in the density of spines (Elston *et al.*, 1997, 2001, 2005a; Elston and Rosa, 1997; Benavides-Piccione *et al.*, 2002; DeFelipe *et al.*, 2002; Elston and DeFelipe, 2002). However, it remains unknown whether this is a general evolutionary trend, or if in small-brained species, e.g. mice, circuits in different cortical areas have similar cellular components. Furthermore, from previous studies it is difficult to determine if these cortical differences represent a systematic gradient of morphological features, as occurs in other parts of the body plan. Also, available data only take into account measurements of individual morphological parameters to evaluate how different or similar two neuronal morphologies are based on measurements of individual morphological parameters. What constitutes two different neuron types to one investigator could become a single group to another.

To rigorously address these questions, in the present study we investigated the basal dendritic arbors of layer II/III pyramidal neurons from three different and distant regions of the mouse neocortex: the secondary motor cortex (M2), the secondary somatosensory cortex (S2), and the lateral secondary visual cortex and association temporal cortex (V2L/TeA), using principal component analysis (PCA)-based cluster analysis of the multidimensional dataset of 156 morphological parameters sampled from three-dimensional reconstructions. To avoid methodological problems, we used an unbiased sampling

method and the same technical conditions for all neurons. We chose to study the basal dendritic arbors in horizontal sections to systematically compare a complete and major dendritic region of the pyramidal neurons, and because the majority of quantitative studies of pyramidal cell structure in the cerebral cortex have been performed on the basal dendritic arbors of layer III in different primate species and cortical areas. We also sampled neurons from the same animals to prevent differences between animals. Using cluster analysis we determined the statistical properties of the neurons in each of the three cortical areas chosen and directly plotted their differences in variance, while keeping track of the exact position of each neuron in the cortex. Our data confirm the existence of systematic morphological differences among neurons of different cortical regions. Moreover, we find that the key difference lies in the size of their dendritic trees. Finally, we cannot account for these differences assuming a simple gradient of sizes across the cortex. The simplest interpretation of our data is that each cortical region is built with different types of pyramidal neurons.

Materials and Methods

Tissue Preparation and Intracellular Injections

BC57 Black mice ($n = 2$, 2 months old) were overdosed by intraperitoneal injection of sodium pentobarbitone and perfused intracardially with 4% paraformaldehyde. Their brains were then removed and the cortex of the right hemisphere flattened between two glass slides (e.g. Welker and Woolsey, 1974) and further immersed in 4% paraformaldehyde for 24 h. Sections (150 μm) were cut parallel to the cortical surface with a Vibratome. By relating these sections to coronal sections we were able to identify, by cytoarchitectural differences, the section that contained layer II/III among the rest of cortical layers allowing the subsequent injection of cells at the base of layer II/III (e.g. see Fig. 3 of Elston and Rosa, 1997). Our cell injection methodology has been described in detail elsewhere (Elston *et al.*, 1997, 2001; Elston and DeFelipe, 2002). Briefly, cells were individually injected with Lucifer Yellow in three different regions of the neocortex [approximately corresponding to areas M2, S2 and V2L/TeA of Franklin and Paxinos (1997)] by continuous current that was applied until the distal tips of each dendrite fluoresced brightly. Following injections, the sections were processed with an antibody to Lucifer Yellow, as described in Elston *et al.* (2001) to visualize the complete morphology of the cells (Fig. 1). Only neurons that had an unambiguous apical dendrite and whose basal dendritic tree was completely filled and contained within the section were included in this analysis.

Reconstruction of Cortical Neurons

The Neurolucida package (MicroBrightField) was used to three-dimensionally trace the basal dendritic arbor of pyramidal cells in each cortical region. All neurons that were judged to be completely filled (as evident by the termination of all their dendritic branches in a normally round tip, far from the plane of section and without any graded loss of stain) were included for analysis. For each reconstructed basal skirt (30 in M2, 30 in S2, 30 in V2L/TeA), we performed the branched structure, convex hull, Sholl, fractal, fan in diagram, vertex, and branch angle analyses (incorporated in the Neurolucida package) and measured a battery of 156 morphological parameters that included features of the basal dendritic tree and the soma (Supplementary Table 1):

- Total number of nodes (branch points) and endings (end or termination points) contained in the basal dendritic arbor.
- Total dendritic length (per cell) and mean length (taking into account the quantity of dendrites) of basal dendritic arbor.

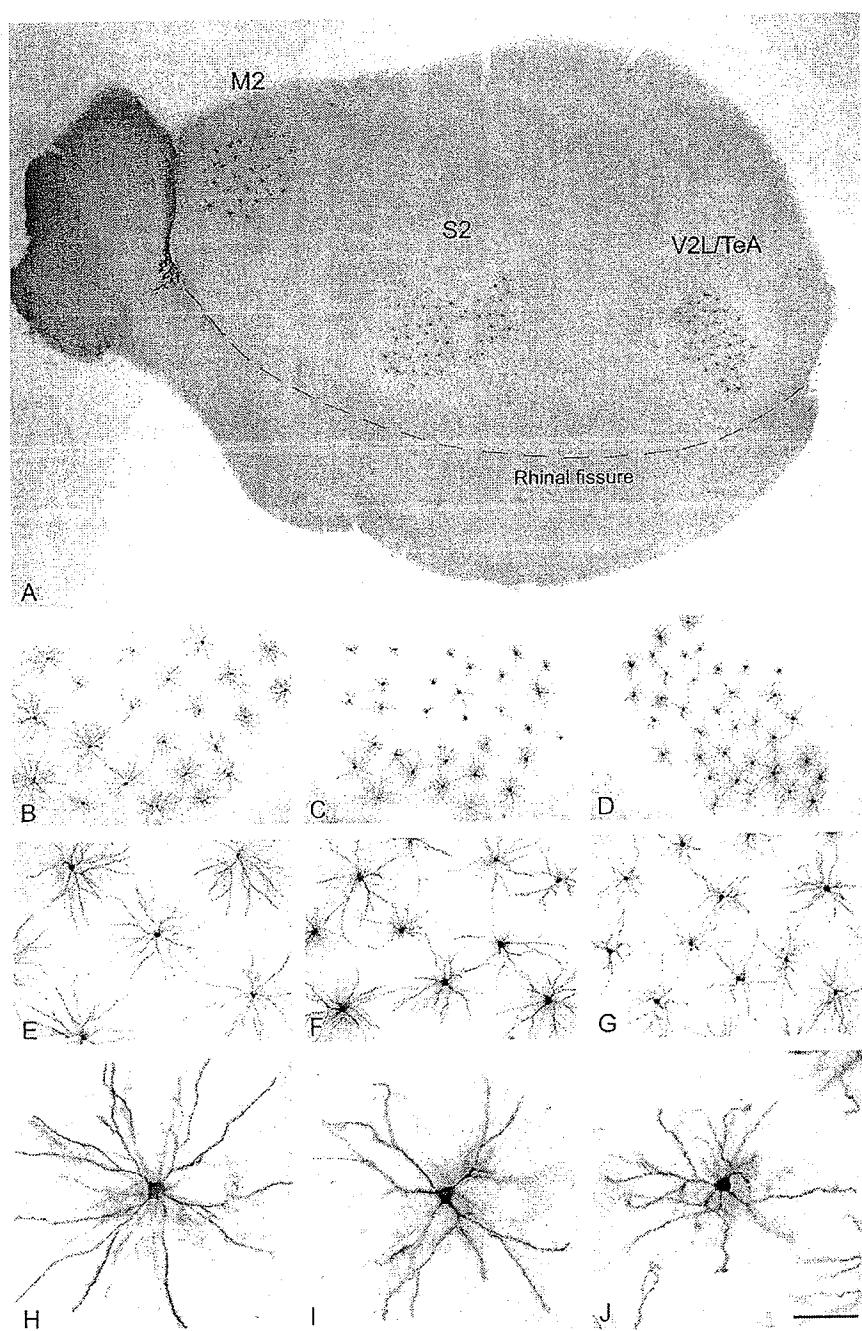


Figure 1. Reconstruction of mouse layer II/III pyramidal cells' basal dendrites. *(A)* Low-power photomicrograph of the mouse cerebral cortex cut parallel to the cortical surface, showing the regions where cells were injected [approximately corresponding to areas M2, S2 and V2L/TeA of Franklin and Paxinos (1997) respectively]. These neurons were injected in layer II/III with Lucifer Yellow and then processed with a light-stable diaminobenzidine. *(B–J)* Successive higher magnification photomicrographs showing pyramidal cells basal dendrites in M2 *(B, E, H)*, S2 *(C, F, I)* and V2L/TeA *(D, G, J)* regions. Scale bar = 815 µm in *A*; 350 µm in *B–D*; 150 µm in *E–G*; 60 µm in *H–J*.

- Basal dendritic field area (BDFA), which measures the area of the dendritic field of a neuron calculated as the area enclosed by a polygon that joins the most distal points of dendritic processes (convex area).
- Somatic aspects, such as length (perimeter), surface area, minimum and maximum feret (which gives information about the shape, and refers to the smallest and largest dimensions of the soma contour as if a caliper had been used to measure across the contour), compactness (which describes the relationship between the area and the

maximum diameter, being the compactness for a circle = 1), convexity (which measures one of the profiles of complexity, being the convexity of circles, ellipses and squares = 1), form factor (which refers to the shape of the contour, being 1 a perfect circle and approaching 0 as the contour shape flattens out; this variable differs from the compactness by considering the complexity of the perimeter of the object), roundness (i.e. the square of the compactness), aspect ratio (which evaluates the degree of flatness as the ratio of its minimum diameter to its maximum diameter),